Validation of a photoacoustic gas analyser for the measurement of functional residual capacity using multiple-breath inert gas washout

Gonem, Sherif ; Singer, Florian ; Corkill, Steven ; Singapuri, Amisha ; Siddiqui, Salman ; Gustafsson, Per

Abstract: BACKGROUND: The respiratory mass spectrometer is the current gold-standard technique for performing multiple-breath inert gas washout (MBW), but is expensive and lacks portability. A number of alternative techniques have recently been described. OBJECTIVES: We aimed to validate, using an in vitro lung model, an open-circuit MBW system that utilises a portable photoacoustic gas analyser, with sulphur hexafluoride (SF6) as the inert tracer gas. METHODS: An acrylic glass lung model was utilised to assess the accuracy of functional residual capacity (FRC) measurements derived from MBW. Measurements were performed in triplicate at 20 combinations of simulated FRC, tidal volume and respiratory rate. FRC measured using MBW (FRCmbw) was compared to FRC calculated from the known dimensions of the model (FRCcalc). MBW was also performed in 10 healthy subjects and 14 patients with asthma. RESULTS: The MBW system measured FRC with high precision. The mean bias of FRCmbw with respect to FRCcalc was -0.4% (95% limits of agreement of -4.6 and 3.9%). The mean coefficient of variation of triplicate FRC measurements was 4.0% in vivo and 1.0% in vitro. MBW slightly underestimated low lung volumes and overestimated high lung volumes, but this did not cause a significant error in lung clearance index except at lung volumes below 1,500 ml. CONCLUSIONS: The open-circuit MBW system utilising SF6 as the inert tracer gas and a photoacoustic gas analyser is both accurate and repeatable within the adult range of lung volumes. Further modifications would be required before its use in young children or infants.

DOI: https://doi.org/10.1159/000357786

Originally published at:
Gonem, Sherif; Singer, Florian; Corkill, Steven; Singapuri, Amisha; Siddiqui, Salman; Gustafsson, Per (2014). Validation of a photoacoustic gas analyser for the measurement of functional residual capacity using multiple-breath inert gas washout. Respiration, 87(6):462-468.
DOI: https://doi.org/10.1159/000357786

Clinical Investigations

Respiration

Respiration 2014;87:462–468
DOI: 10.1159/000357786

Received: August 7, 2013
Accepted after revision: December 3, 2013
Published online: February 13, 2014

Validation of a Photoacoustic Gas Analyser for the Measurement of Functional Residual Capacity Using Multiple-Breath Inert Gas Washout

Sherif Gonem[a] Florian Singer[b] Steven Corkill[a] Amisha Singapuri[a]
Salman Siddiqui[a] Per Gustafsson[c]

[a]Institute for Lung Health, University of Leicester, Leicester, UK; [b]University Children's Hospital of Zurich, Zurich, Switzerland; [c]Department of Paediatrics, Central Hospital, Skövde, Sweden

For editorial comment see p. 456

Key Words
Pulmonary physiology · Validation · Functional residual capacity

Abstract
Background: The respiratory mass spectrometer is the current gold-standard technique for performing multiple-breath inert gas washout (MBW), but is expensive and lacks portability. A number of alternative techniques have recently been described. ***Objectives:*** We aimed to validate, using an in vitro lung model, an open-circuit MBW system that utilises a portable photoacoustic gas analyser, with sulphur hexafluoride (SF_6) as the inert tracer gas. ***Methods:*** An acrylic glass lung model was utilised to assess the accuracy of functional residual capacity (FRC) measurements derived from MBW. Measurements were performed in triplicate at 20 combinations of simulated FRC, tidal volume and respiratory rate. FRC measured using MBW (FRC_{mbw}) was compared to FRC calculated from the known dimensions of the model (FRC_{calc}). MBW was also performed in 10 healthy subjects and 14 patients with asthma. ***Results:*** The MBW system measured FRC with high precision. The mean bias of FRC_{mbw} with respect to FRC_{calc} was –0.4% (95% limits of agreement of –4.6 and 3.9%). The mean coefficient of variation of triplicate FRC measurements was 4.0% in vivo and 1.0% in vitro. MBW slightly underestimated low lung volumes and overestimated high lung volumes, but this did not cause a significant error in lung clearance index except at lung volumes below 1,500 ml. ***Conclusions:*** The open-circuit MBW system utilising SF_6 as the inert tracer gas and a photoacoustic gas analyser is both accurate and repeatable within the adult range of lung volumes. Further modifications would be required before its use in young children or infants.

Introduction

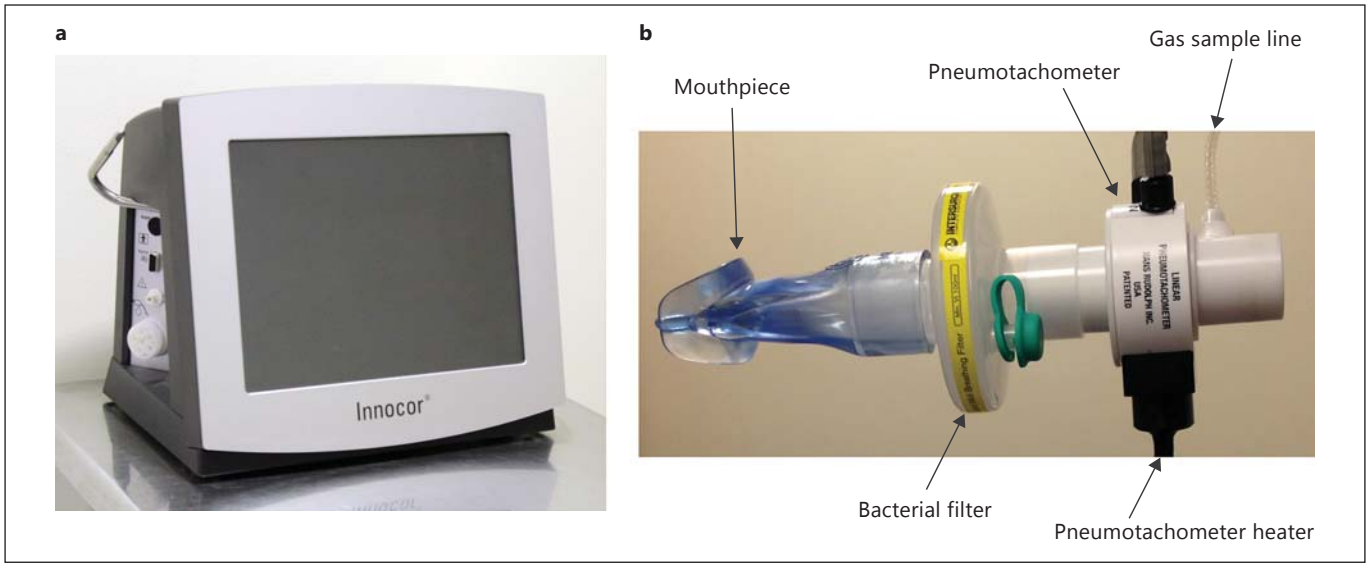
Multiple-breath inert gas washout (MBW) is a technique for assessing the non-uniformity of ventilation distribution in the lungs by measuring the efficiency with which an inert tracer gas is washed out of the lungs [1]. The current gold-standard MBW system is the respiratory mass spectrometer [1], but this is expensive and lacks portability. An alternative system based upon a modified photoacoustic gas analyser (Innocor™, Innovision A/S, Odense, Denmark) and 0.2% sulphur hexafluoride (SF_6) as the tracer gas has been developed, and shown to be both repeatable and practical [2], but its accuracy has not been formally validated. The functional residual capacity (FRC) may be derived from an MBW by dividing the total volume of inert gas expired by the difference between the inert gas concentrations at the beginning and end of the washout period [3]. Current guidelines recommend that MBW systems are validated by determining FRC mea-

KARGER

E-Mail karger@karger.com
www.karger.com/res

0025–7931/14/0876–0462$39.50/0

Dr. Sherif Gonem
Respiratory BRU
Glenfield Hospital
Leicester, LE3 9QP (UK)
E-Mail sg330@le.ac.uk



Color version available online

Fig. 1. Photoacoustic gas analyser (**a**) and patient interface (**b**).

surement accuracy, and in particular that measured FRC values should lie within 5% of known volumes, at least 95% of the time [1]. Accurate FRC determination depends critically upon correct flow and gas concentration measurements, precise synchronisation of these signals, and adequate conversion of measured flows and volumes to body temperature, pressure and water vapour saturation (BTPS) conditions. These are the same technical factors that influence the accuracy of clinically relevant parameters such as the lung clearance index (LCI). FRC is a suitable end-point for quality control and methodological validation because measured values may be readily compared to a gold standard, such as the known volume of an in vitro lung model. Current guidelines [1] recommend that MBW systems are validated using in vitro lung models incorporating realistic BTPS conditions, as previously described by Singer et al. [4]. We aimed to utilise this lung model to validate the MBW method of Horsley et al. [2], as well as to compare the variability of triplicate MBW tests performed in vitro and in vivo. The primary outcome of the study was the percentage difference between measured and calculated FRC measurements using the in vitro lung model, with satisfactory accuracy being defined as a percentage difference of between –5 and 5% for at least 95% of the measurements. Secondary outcomes were the dependence, if any, of the measurement bias on absolute FRC values or the respiratory rate, as well as the difference in measurement variability between in vitro and in vivo measurements.

Materials and Methods

Description of the Modified Innocor Photoacoustic Gas Analyser

The Innocor photoacoustic gas analyser is a small, portable device which weighs approximately 8 kg and has dimensions of 35 × 29 × 26 cm. It was originally designed to measure cardiac output at rest and during exercise, by measuring the difference in absorption between N_2O, which crosses the alveolar-capillary membrane, and SF_6, which does not. In addition, the device can simultaneously measure oxygen uptake and carbon dioxide production. At the heart of the Innocor device is a photoacoustic gas analyser, which is capable of measuring the concentrations of CO_2, SF_6 and N_2O at high accuracy and temporal resolution. A separate oxygen analyser is placed in series before the photoacoustic analyser. A photograph of the device is shown in figure 1a.

The Innocor device has recently been modified for the performance of MBW [2], and we modified our device in a similar manner. Firstly, the commercially supplied patient interface has an excessive dead space for inert gas washout, and was therefore replaced with a mesh-type pneumotachometer (3,700 series, Hans Rudolph Inc., Kansas City, Mo., USA), heated to 37°C using a 3850A series pneumotachometer heater (Hans Rudolph Inc.). The patient interface, shown in figure 1b, comprised a rubber mouthpiece connected to a bacterial filter (Clear-Guard Midi, Intersurgical, Wokingham, UK), which was in turn connected to the pneumotachometer. The gas sample needle was positioned distal to the pneumotachometer mesh, resulting in a minimal post-capillary dead space of 2.65 ml. The pre-capillary dead space was calculated as 40 ml for in vitro tests and 54.6 ml for in vivo tests (due to the additional dead space of the rubber mouthpiece). A further modification made to our Innocor device was to manually bypass the oxygen analyser by diverting the internal Nafion tubing directly into the photoacoustic analyser. This had the effect of reducing both the gas analyser rise time and the flow-gas delay time.

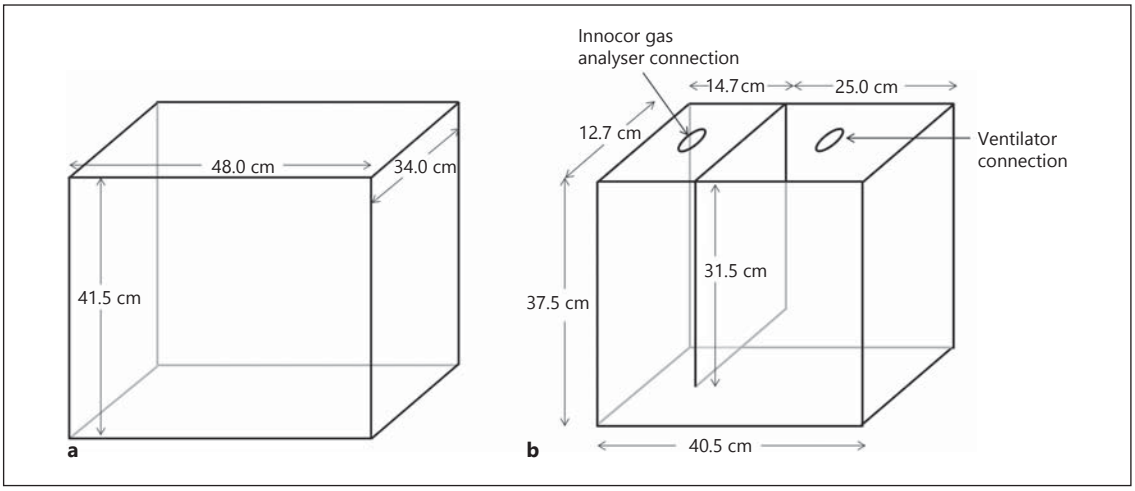


Fig. 2. Schematic diagram of the lung model showing the dimensions of the outer (**a**) and inner (**b**) tanks.

Description of the Lung Model

We used a simple one-compartment model of the lung consisting of an enclosed clear acrylic glass tank (Soloplex, Tidaholm, Sweden), partly filled with water at 37ºC, as previously described [4]. In order to achieve BTPS conditions within the phantom lung, it was enclosed within a further acrylic glass tank containing water that was kept at a constant temperature of 37°C by a thermostat. The dimensions of the outer and inner tanks are shown in figure 2. The inner tank was fitted with an off-centre vertical partition that divided it into a larger and a smaller section. The partition did not reach the base of the tank, thus allowing the two sides to communicate, but the water level was always kept above the lower end of the partition. The lid of the larger section was connected to a bi-level positive airway pressure ventilator (Vivo 30, Breas Medical AB, Mölnlycke, Sweden) which exerted alternating high and low pressures on the water surface, designated inspiratory and expiratory positive airway pressures (IPAP and EPAP), respectively. Due to the communication below the partition, this caused the water level in the smaller section to alternately rise and fall, simulating diaphragmatic movement. The IPAP setting on the ventilator determined the FRC of the phantom lung, whilst the difference between the IPAP and EPAP settings determined the tidal volume (VT). The lid above the smaller section was modified to fit the patient interface described above, with the exception that the rubber mouthpiece was omitted and the lung model attached directly to the bacterial filter.

In vitro Testing Procedure

Before testing began, a volume calibration was performed using a 1-litre syringe at low, medium and high flow rates. The Innocor gas analyser had recently been serviced, including a gas calibration. All tests were performed on the same day (December 17, 2011), and the air temperature and relative humidity inside the phantom lung were measured on three occasions during the testing period to confirm that BTPS conditions in the lung compartment were maintained. This was performed using measuring probes that were inserted through a small hole in the lung phantom lid. The hole was then sealed prior to testing.

Testing was performed at FRC values between 500 and 4,000 ml, at 500-ml intervals, in order to simulate lung volumes of young children through to large adults. VT was set to approximately one third of FRC. The respiratory rate was set at between 12 and 24 breaths/min; larger respiratory rates were used with smaller lung volumes in order to accurately simulate the physiology of young children. At FRC values of 500, 1,000, 2,000, 3,000 and 4,000 ml, experiments were performed at three different respiratory rates in order to assess if accuracy was affected by changes in this parameter. Each of the 20 experiments was performed in triplicate, making a total of 60 washout runs.

The testing procedure consisted of the following steps.

(i) The water level, IPAP and EPAP settings were adjusted to achieve the required FRC and VT within the phantom lung. This was facilitated by a measuring scale affixed to the inside of the lung model, which was utilised to measure the water level at its highest and lowest points during the respiratory cycle. The respiratory rate was set using the ventilator controls.

(ii) An air mixture containing 0.2% SF_6 was passively insufflated into the lung phantom via an open-circuit bypass flow system, and expiratory SF_6 concentration was monitored online using the Innocor gas analyser. The flow rate of SF_6 through the open circuit was increased sufficiently to allow complete wash-in of SF_6.

(iii) Once complete equilibration (wash-in) had occurred, meaning that the inspired and expired SF_6 concentrations were equal, the bypass flow system was removed so that the phantom lung inspired from ambient air. Expiratory SF_6 concentration continued to be monitored during the wash-out phase, and the experiment was terminated once the expired SF_6 concentration fell below 0.005% (1/40th of the initial value) for three consecutive breaths.

Subjects

Ten healthy subjects with no history of respiratory disease and 14 patients with a clinical diagnosis of asthma were recruited. Subjects were aged over 18 years, and were never-smokers or ex-smokers with ≤10 pack years of smoking history. The study protocol was approved by the National Research Ethics Committee, East Midlands Leicester (approval No. 08/H0406/189), and all subjects gave their written informed consent.

In vivo Testing Procedure

MBW was performed in triplicate at a single visit between the dates of June 15, 2010, and March 2, 2011, using the method initially described by Horsley et al. [2]. Participants wore a nose clip and breathed an air mixture containing 0.2% SF_6, via an open-circuit bypass flow system connected to an Innocor gas analyser, until the concentration in their exhaled breath reached a steady state. For in vivo tests a Douglas bag, fitted with a two-way valve, was utilised as a reservoir for SF_6. Participants were encouraged to maintain a steady respiratory rate of approximately 12 breaths/min, and a constant VT volume of 1 litre [5], using a real-time visual display of inspired volume as a guide. Participants were then switched to breathing room air during an expiration and asked to continue breathing at the same respiratory rate and VT. The end-tidal concentration of SF_6 in exhaled breath (C_{et}) was recorded during this washout phase until it reached 1/40th of the original concentration (0.005%).

Data and Statistical Analysis

The washout data were transferred to a laptop computer, where they were processed and analysed using custom software written with TestPoint (Measurement Computing Corp., Norton, Mass., USA). This included a calculation of the flow-gas delay time, which was used to synchronise the flow and SF_6 concentration signals. We calculated the flow-gas delay time using the method based upon re-inspiration of SF_6 from the post-capillary dead space, as described in recent guidelines [1]. Specifically, the time point at which the post-capillary dead space had been inspired was aligned with the time point at which the inspired SF_6 concentration fell to 50% of its initial value. FRC was calculated by dividing the total volume of SF_6 expired by the difference between the SF_6 concentrations at the beginning and end of the washout period [3]. The total volume of expired SF_6 was calculated by integrating flow and SF_6 concentration over the course of each expiration, and subtracting the re-inspired SF_6 volume, which was in turn calculated by integrating flow and SF_6 concentration over the course of each inspiration. LCI was defined as the cumulative expired volume at the point at which C_{et} fell to 1/40th of its initial value, divided by the FRC [6].

Statistical analyses were performed using Prism version 6 (GraphPad, San Diego, Calif., USA). Results from the in vitro lung model were displayed as a Bland-Altman plot [7] of FRC measured using MBW (FRC_{mbw}) against FRC calculated using the measuring scale affixed to the lung model (FRC_{calc}). The percentage difference between FRC_{mbw} and FRC_{calc} was also compared between washout tests with low, intermediate and high respiratory rates, using one-way analysis of variance. The mean coefficient of variation (CoV) of triplicate values was compared across the three sets of measurements (lung model, healthy subjects and patients with asthma) using one-way analysis of variance with Bonferroni correction for multiple comparisons.

Results

The Innocor system measured FRC with high precision. Table 1 lists the 60 in vitro experiments that were performed, with the values of FRC_{calc} and FRC_{mbw} given in each case. Figure 3 shows Bland-Altman plots [7] of FRC_{mbw} against FRC_{calc}, with the absolute and percentage difference between FRC_{mbw} and FRC_{calc} plotted on the y-axis. The mean absolute bias of FRC_{mbw} with respect to FRC_{calc} was 12.6 ml, and the 95% limits of agreement were –68.6 and 93.7 ml. The mean percentage bias of FRC_{mbw} with respect to FRC_{calc} was –0.4%, and the 95% limits of agreement were –4.6 and 3.9%. There was a significant positive correlation between the mean of FRC_{mbw} and FRC_{calc} and the percentage difference between these values (Pearson correlation coefficient = 0.82, $p < 0.0001$). The equation of the regression line was $y = 0.001462x - 3.653$. The regression line crossed the x-axis at a lung volume of 2,499 ml, which was therefore the point of zero bias. The mean bias at a lung volume of 500 ml was –3.9% and at a lung volume of 4,000 ml was 1.9%. The respiratory rate did not have an independent effect on the bias. The mean bias of FRC_{mbw} with respect to FRC_{calc} in washout runs with low, intermediate and high respiratory rates was –0.3, –0.9 and –0.3%, respectively, with no statistically significant difference between the sets of washout experiments. Figure 4 shows the error in LCI, defined as the absolute difference between the measured and the calculated LCI, against FRC_{calc}. LCI was measured accurately at lung volumes at or above 1,500 ml, with an error of less than 0.4 units in each case. However,

Table 1. List of experiments performed with results

Experimental conditions			FRC_{mbw} results, ml		
VT, ml	respiratory rate, min^{-1}	FRC_{calc}, ml	Test 1	Test 2	Test 3
224	16	477	460	467	462
205	20	496	465	474	475
187	24	496	478	486	473
243	16	944	902	917	922
261	24	1,019	1,006	995	1,009
336	20	1,093	1,077	1,097	1,077
448	16	1,467	1,449	1,464	1,476
523	12	1,971	1,925	1,997	2,001
504	16	1,971	1,936	1,981	1,960
467	20	2,008	2,020	2,016	2,038
541	12	2,550	2,512	2,551	2,543
1,027	12	2,587	2,558	2,606	2,610
990	20	2,998	3,039	3,020	3,015
1,046	16	2,998	3,056	3,008	2,998
485	12	3,035	3,045	3,097	3,108
1,008	12	3,072	3,068	3,084	3,096
448	12	3,577	3,599	3,631	3,595
1,008	12	3,950	4,100	3,985	4,072
971	16	3,987	3,990	4,046	4,091
952	20	4,006	4,047	4,135	4,050

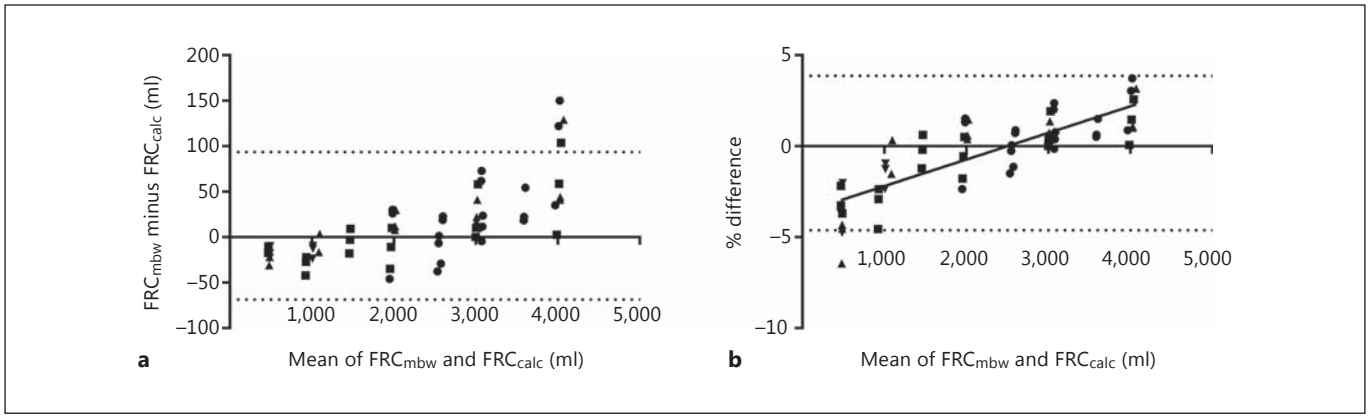


Fig. 3. Bland-Altman plots of FRC_{mbw} against FRC_{calc}, with absolute differences (**a**) and percentage differences (**b**). The percentage difference is calculated as $100(FRC_{mbw} - FRC_{calc})$ divided by the mean of FRC_{mbw} and FRC_{calc}. Dotted lines represent 95% limits of agreement. The best-fit linear regression line is shown in **b** (Pearson correlation coefficient = 0.82, $p < 0.0001$). Washout runs were with respiratory rates of 12 (●), 16 (■), 20 (▲) and 24 (▼) breaths/min.

at lung volumes of 500 or 1,000 ml, LCI was in some cases overestimated by up to 1.1 units.

The healthy participants comprised 4 women and 6 men with a mean age of 46.4 years (SD 19.4), while the patients with asthma comprised 7 women and 7 men with a mean age of 59.0 years (11.9). The mean FRC_{mbw} was 2,796 ml (896) in healthy controls and 2,555 ml (794) in patients with asthma. The mean LCI was 7.07 (1.07) in healthy controls and 8.12 (1.46) in patients with asthma.

The mean CoV of triplicate FRC_{mbw} measurements was 1.0% (range 0.4–2.2) in vitro, 4.0% (1.9–5.3) in healthy controls and 2.9% (0.4–5.4) in patients with asthma. These values were significantly different across the three sets of measurements ($p < 0.0001$). In particular, the mean CoV was significantly lower in vitro than in both healthy ($p < 0.0001$) and asthma ($p < 0.001$) groups, but did not differ significantly between the healthy and asthma groups. The difference in mean CoV between in vitro and healthy group measurements was 3.0% (95% confidence interval of difference: 2.5–3.5%), while the difference in mean CoV between in vitro and asthma group measurements was 1.9% (95% confidence interval of difference: 1.0–2.7%). The in vitro CoV of FRC_{mbw} was not significantly related to the respiratory rate. The mean CoV was 1.1% (0.5–2.2) with a respiratory rate of 12 breaths/min, 1.0% (0.8–1.3) with a respiratory rate of 16 breaths/min, and 0.9% (0.4–1.4) with a respiratory rate of 20 or 24 breaths/min (no significant difference between groups of tests). The mean CoV of triplicate LCI measurements was 1.2% (0.0–4.0) in vitro, 4.6% (0.4–12.1) in healthy controls, and 3.3% (0.3–7.0) in patients with asthma.

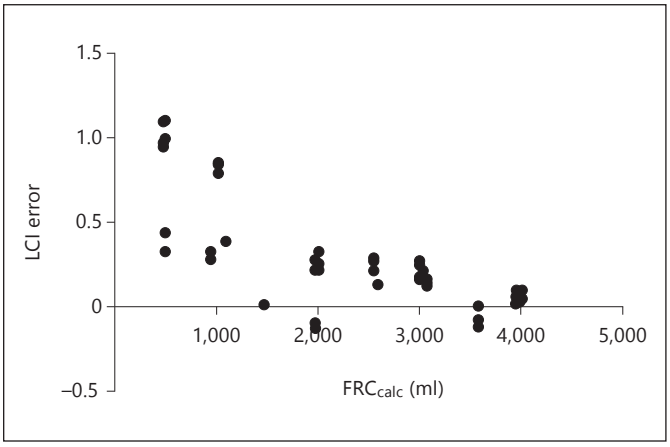


Fig. 4. Error in LCI against calculated FRC.

Discussion

In this study, we utilised a one-compartment acrylic glass lung model under BTPS conditions to validate a practical and portable MBW system that uses an Innocor photoacoustic gas analyser, with SF_6 as the inert tracer gas. Of note, this open-circuit system is distinct from the closed-circuit setup that was found to have poor intra-subject variability and patient acceptability by Pittman et al. [8]. We found good agreement between FRC measured using Innocor (FRC_{mbw}) and FRC calculated from the known dimensions of the lung model (FRC_{calc}), with a mean bias of FRC_{mbw} with respect to FRC_{calc} of –0.4%, and 95% limits of agreement of –4.6

and 3.9%, comfortably below the recommended maximum error of 5% [1]. An identical lung model has been previously used to validate a commercially available open-circuit nitrogen MBW system (Exhalyzer D™, Eco Medics AG, Duernten Switzerland) [4]. These authors found that for lung volumes above 500 ml, there was a mean bias of 0.4%, with 95% limits of agreement of –4.0 and 4.7%, respectively. We therefore conclude that the accuracy of FRC measurements using Innocor and Exhalyzer D is similar within the FRC range of 500–4,000 ml. We found that the mean coefficient of variation of triplicate FRC measurements was 1.0% in vitro and 4.0% in vivo, suggesting that the majority of between-measurement variability in vivo was caused by biological rather than technical factors. Singer et al. [4] obtained similar values using the Exhalyzer D system, namely 1.4 and 4.5%, respectively, suggesting that the two systems perform approximately equally with respect to within-visit repeatability.

The Innocor-based system appeared to slightly underestimate FRC when lung volumes were small and overestimate it when volumes were large. We speculated that this may have been due to cyclical heating and cooling of the pneumotachometer during the respiratory cycle, resulting in non-linearity of flow measurement. On closer examination of our data, we ascertained that the source of this FRC-dependent bias was the correction for re-inspired SF_6, which appeared to overcompensate at low lung volumes, resulting in artificially low FRC values. In particular, inspiratory flows were overestimated at low lung volumes, particularly below an FRC of 1,500 ml, resulting in an overestimation of re-inspired SF_6 volume. Of note, this FRC-dependent bias was not seen with the Exhalyzer D [4], which utilises an ultrasonic flowmeter. However, the error in FRC measurements with Exhalyzer D increased with increasing respiratory rate [4], an effect that we did not observe with the Innocor-based system. We further examined the accuracy of LCI measurements performed using Innocor at different lung volumes and found that at lung volumes of 1,500 ml or above, there was good agreement between measured and calculated LCI, whereas at lung volumes below 1,500 ml, LCI was often overestimated by up to 1.1 units. This suggests that at lung volumes corresponding to older children or adults, the small bias observed in FRC does not significantly affect LCI measurements.

The open-circuit Innocor-based system described in this manuscript is practical and convenient, and could potentially be utilised in clinical practice and multicentre trials in both older children and adults. However, our in vitro results at low lung volumes suggest that the system would require further modification before it could be used reliably in young children and infants. Such modification would be likely to include the replacement of the pneumotachometer with a smaller model that has a lower flow range. Moreover, Innocor employs side-stream sampling of gas at a flow rate of 120 ml/min [1], which may have a significant influence at low VTs. Furthermore, the response time of the photoacoustic analyser is relatively slow (154 ms) [1], which may be particularly relevant at fast respiratory rates, as seen in young children. These latter two issues would require further technical development by the manufacturers of Innocor. There are a number of additional improvements that could be made relatively easily to further increase the general applicability of this technology to the performance of MBW. Foremost among these is that data analysis is currently performed off-line, which is relatively time consuming. It would, however, be straightforward to incorporate FRC and LCI calculations into the on-board Innocor software in the future. Ideally, this on-board software would also include a user-friendly patient interface to allow VT to be targeted by the patient to a set value. Our current system requires the patient to target their VT using a numerical display on a separate laptop computer. A further limitation of the current Innocor setup is the requirement of SF_6, which is restricted in some countries as it acts as a greenhouse gas.

In conclusion, the open-circuit MBW system utilising SF_6 as the inert tracer gas and an Innocor photoacoustic gas analyser is both accurate and repeatable in adults, and is comparable in these respects to the Exhalyzer D MBW system. These results provide increased confidence in previous and future research studies conducted using the Innocor-based system, and suggest its potential to develop into a commercially available MBW platform. Further modifications to the system would be required to facilitate its use in young children and infants.

Acknowledgements

The research leading to these results has received funding from the European Union Seventh Framework Programme FP7/2007–2013 under grant agreement No. 270194, as part of the Airway Disease Predicting Outcomes through Patient Specific Computational Modelling (AirPROM) project (www.airprom.eu). Florian Singer was supported by the European Respiratory Society, STRTF fellowship No. 81-2011.

References

1 Robinson P, Latzin P, Verbanck S, Hall GL, Horsley A, Gappa M, Thamrin C, Arets HG, Aurora P, Fuchs S, King GG, Lum S, Macleod K, Paiva M, Pillow J, Ranganathan S, Ratjen F, Singer F, Sonnappa S, Stocks J, Subbarao P, Thompson B, Gustafsson PM: Consensus statement for inert gas washout measurement using multiple and single breath tests. Eur Respir J 2013;41:507–522.

2 Horsley AR, Gustafsson PM, Macleod KA, Saunders C, Greening AP, Porteous DJ, Davies JC, Cunningham S, Alton EW, Innes JA: Lung clearance index is a sensitive, repeatable and practical measure of airways disease in adults with cystic fibrosis. Thorax 2008;63:135–140.

3 Darling RC, Cournand A, Richards DW: Studies on the intrapulmonary mixture of gases. III. An open circuit method for measuring residual air. J Clin Invest 1940;19:609–618.

4 Singer F, Houltz B, Latzin P, Robinson P, Gustafsson P: A realistic validation study of a new nitrogen multiple-breath washout system. PLoS One 2012;7:e36083.

5 Verbanck S, Schuermans D, van Muylem A, Noppen M, Paiva M, Vincken W: Ventilation distribution during histamine provocation. J Appl Physiol 1997;83:1907–1916.

6 Bouhuys A, Lichtneckert S, Lundgren C, Lundin G: Voluntary changes in breathing pattern and N2 clearance from lungs. J Appl Physiol 1961;16:1039–1042.

7 Bland JM, Altman DG: Statistical methods for assessing agreement between two methods of clinical measurement. Lancet 1986;1:307–310.

8 Pittman JE, Johnson RC, Jones PW, Davis SD: Variability of a closed, rebreathing setup for multiple breath wash-out testing in children. Pediatr Pulmonol 2012;47:1242–1250.